REMARKS

Applicants have amended the title and abstract to more accurately reflect the claimed invention. In addition, Applicants have amended the paragraph beginning on page 1, line 1 (added by Preliminary Amendment on March 31, 2004) to alter the priority claim. Specifically, Applicants have deleted the priority claim to U.S Application Number 07/289,589, filed December 23, 1988. This application now claims priority to December 22, 1989.

Claims 60-62 are pending. Claims 1-47 have been cancelled. Claims 48-59 and 63-72 have been withdrawn as a result of a restriction requirement and species election. The claims have been amended or cancelled without any intention to abandon the subject matter of those claims as filed or later amended, but with the intention that the claims of the same, greater, or lesser scope may be pursued in a continuation application.

Applicants have amended claim 60 to recite the elements of method claim 48, from which it formerly depended. Claims 60 and 61 have also been amended to specify that the claimed compositions comprise "human" glucocerebrosidase. Support for this amendment may be found at page 1, line 23 to page 2, line 6 of the specification which refers to human glucocerebrosidase protein, the amino acid sequence of which was known. Claims 60 and 61 have also been amended to recite a "pharmaceutical" composition "suitable for the treatment of a human patient having Gaucher's disease...." This amendment is supported at page 5, lines 17-20. No new matter has been added.

Interview

Applicants would like to thank the Examiner for the interview conducted on October 18, 2005, including his careful consideration of the application and helpful discussion of the issues raised in the Office Action. During the interview, the rejections and cited references were reviewed and amendments to overcome these were discussed. Applicants believe that the claim amendments overcome the outstanding rejections thereby placing this case in condition for immediate allowance.

Rejection of claims under 35 USC § 112, First paragraph – Written description

Claims 60-62 were rejected under 35 USC § 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner asserts that the specification defines "glucocerebrosidase" as "all enzymes having an enzyme activity which causes hydrolysis of a glucocerebroside" and thus that "the claims encompass a highly divergent genus encompassing any polypeptide capable of hydrolyzing a glyucocerebroside isolated from any mammalian cell wherein conversion of $Glc_3Man_9GlcNac_2$ to smaller species has been inhibited."

Applicants have amended claims 60 and 61 to specify that the claimed compositions comprise "human" glucocerebrosidase. It is glucocerebrosidase having the amino acid sequence of the glucocerebrosidase enzyme found in humans that Applicants intend to cover and the claims have been so limited.

The Examiner also relies on Houdebine et al., Transgen. Res., 9:305-320 (1986) (hereinafter "Houdebine") as teaching that "proper post-translation processing of proteins expressed at pharmaceutical levels is often unpredictable because the mechanisms are dependent on cellular enzymes that are present at variable concentration in different cell types."

Houdebine is directed toward the production of recombinant proteins in transgenic animals *in vivo*. In particular, Houdebine states that recombinant proteins produced in the milk of transgenic animals (i.e., by mammary cells) are not always glycosylated in an appropriate manner.

In contrast to transgenic production *in vivo*, where glycosylation processes are not particularly amenable to control, Applicants' claimed invention is directed toward production of glucocerebrosidase *in vitro*. Thus, Applicants' invention allows one to control the glycosylation process in cell culture rather than having it depend on the cell type used for expression *in vivo*.

Specifically, treatment of cultured cells capable of expressing human glucocerebrosidase with inhibitors of carbohydrate processing that act to inhibit the conversion of $Glc_3Man_9GlcNac_2$ to smaller species leads to production of glucocerebrosidase having exposed mannose residues. As taught by the specification, glucocerebrosidase with exposed mannose residues has a higher affinity for the human

mannose receptor. It is well known in the art that binding of glucocerebrosidase to the human mannose receptor is critical for its uptake by target cells. Glucocerebrosidase produced in accordance with the invention is therefore particularly useful for the treatment of Gaucher's disease.

Moreover, the specification teaches several methods for analyzing the sugar structures on glucocerebrosidase and teaches methods to determine the ability of glucocerebrosidase to bind to and be taken up by macrophages. Thus, given the teachings of the specification, the skilled artisan could readily practice the claimed invention with a variety of mammalian cell types in order to produce human glucocerebrosidase suitable for the treatment of a human patient having Gaucher's disease.

Rejection of claims under 35 USC § 112, First paragraph – Enablement

Claims 60-62 were rejected under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement. Specifically, the Examiner asserts that the art does not disclose the structural features of the "essentially unlimited" genus of any polypeptide capable of hydrolyzing a glucocerebroside and that glycosylation in mammalian cells is unpredictable. The Examiner further asserts that there was no established enzyme replacement therapy for Gaucher's disease at the time the application was filed. Specifically, the Examiner relies on Beutler et al., Blood, 78(5):1183-89 (1991) (hereinafter "Beutler") as teaching that "although a number of attempts to treat Gaucher disease by enzyme replacement were made in the 1970's, these were unsuccessful...." The Examiner acknowledges that Beutler discloses an enabled enzyme replacement method, but asserts that the disclosure of Beutler was not available to the skilled artisan as of Applicants' filing date. The Examiner concludes that the skilled artisan would not be able to make and use the claimed invention without undue experimentation.

As explained above, Applicants have amended the claims to specify that the compositions comprise "human" glucocerebrosidase. The relative predictability of glycosylation using the methods of Applicants' invention has been discussed above.

Regarding Beutler, it states that the early attempts to treat Gaucher's disease by enzyme replacement were "probably doomed to failure partly because of an inadequate supply of enzyme." Given the disclosure of the specification, supply would not have been an issue. Furthermore, Beutler acknowledges that modification of the enzyme to expose mannose residues would presumably improve targeting to macrophages (see page 1183, 2nd paragraph). Finally, Beutler discusses numerous papers published prior to Applicants' filing date that disclose treatment protocols for Gaucher's disease. In fact, Beutler characterizes two of these papers, both of which deal with treatment using mannose terminated glucocerebrosidase, as being "much more encouraging" than certain of the earlier studies (see page 1188, first full paragraph).

Thus, given that treatment protocols were available in the art as of Applicants' filing date, given that supply was not an issue, and given that providing glucocerebrosidase with exposed mannose residues is addressed by Applicants' claimed invention, the skilled artisan would have been able to combine the teachings of the specification with the knowledge available in the art to make and use Applicants' claimed compositions without undue experimentation.

Obviousness-Type Double Patenting

Claims 60-62 were rejected under the judicially created doctrine of obvious-type double patenting over claim 1 of U.S. Patent Number 6,451,600. Applicants submit herewith a Terminal Disclaimer disclaiming the portion of the term of any patent issuing from this application which extends beyond that of the '600 patent.

Rejection of claims under 35 USC § 102

Claims 60-62 were rejected under 35 USC § 102 as being anticipated by Aerts et al, Biochem. Biophys. Res. Commun., 141(2):452-458 (1986) (hereinafter "Aerts"). Specifically, the Examiner states that Aerts teaches "culturing U937 cells in the presence of deoxy-mannojirimycin and recovering a glucocerebrosidase from the cultured cells." In view of the amendments to the claims, Applicants request the rejection be withdrawn.

At the outset, Applicants note that Aerts is not directed to uptake of glucocerebrosidase from the extracellular environment, and in fact, it does not even

mention Gaucher Disease. Aerts is an academic research article directed toward *intracellular* transport of glucocerebrosidase to the lysosome. Nevertheless, Aerts did treat U937 cells with deoxymannojirimycin or swainsonine, homogenized the cells, fractionated the homogenate on a Percoll gradient, and presumably "recovered" fractions containing human glucocerebrosidase.

However, the amended claims recite a "pharmaceutical" composition "suitable for the treatment of a human patient having Gaucher's disease...." The human glucocerebrosidase "recovered" by Aerts doesn't come close to meeting these limitations.

First, Aerts' fractions would not have been "suitable for treatment of a human patient having Gaucher's disease" because they would not have been effective for that purpose. Specifically, the glucocerebrosidase contained within the fractions would not have been taken up by the target cells in Gaucher patients because it would have been hidden within intact cellular organelles. This is evident from Aerts' protocol and results.

Recall that Aerts was studying *intracellular* localization and transport of glucocerebrosidase. To do that, Aerts gently homogenized the cells and fractionated the homogenate on a Percoll gradient. The purpose of using this procedure is to preserve the integrity of the cellular organelles which contain the processing machinery of the cell and effectuate the transport process. This procedure allows one to reproduce the cellular localization of proteins prior to disruption of the cell.

For example, glucocerebrosidase is normally transported to the lysosome by being routed through the golgi apparatus, where it is surrounded with membranes that eventually fuse with the lysosome. The membranes of the golgi, and the glucocerebrosidase contained within them, would have been preserved in Aerts' fractions, as would the lysosomes. That this is the case is evident, for example, from page 455-56 of Aerts, which states that in treated cells, the glucocerebrosidase accumulated in fractions with galactosyltransferase activity, which is a marker of the golgi apparatus. These findings led Aerts to conclude that intracellular transport of glucocerebrosidase to the lysosome was inhibited in cells cultured in the presence of deoxymannojirimycin and swainsonine.

Thus, although glucocerebrosidase protein may have been present in certain of Aerts' fractions, it was not present simply as one of a mixture of isolated proteins.

Rather, it was contained *within* cellular organelles. Therefore, even if Aerts' fractions had been administered to a human patient having Gaucher's disease, the glucocerebrosidase would not have been taken up by the patient's target cells because it would have been hidden. The otherwise "exposed" mannose residues on the surface of the human glucocerebrosidase simply would not have been recognized by the mannose receptors on the patients' target cells. For this reason, Aerts' fractions would not have been useful or suitable for treatment of a human patient having Gaucher's disease.

Additionally, Aerts' fractions would have contained a large number of other proteins derived from the U937 cells used to express the glucocerebrosidase. If injected into a patient, it is likely that the many of these proteins would have been immunogenic and capable of causing a severe immune reaction in patients, thus preventing re-administration of the same therapy. An enzyme composition that could not be re-administered would not be suitable for treatment of a human patient having Gaucher's disease because such patients must be re-treated on a regular basis due to enzyme turnover in the body.

Lastly, it is highly unlikely that any responsible regulatory authority would permit the crude, uncharacterized fractions of Aerts to be administered to human patients. For this reason too, the fractions of Aerts would not be "suitable for the treatment of a human patient having Gaucher's disease."

CONCLUSION

In view of the amendments to the claims and the foregoing remarks, Applicants request that the rejections be reconsidered and withdrawn. In addition, if the product claims are found allowable, Applicants request that the withdrawn process claims be rejoined in accordance with the provisions of MPEP § 821.04, and that claims drawn to non-elected species be considered. If the Examiner believes that a conversation with Applicants' attorney would be helpful in expediting prosecution of this application, he is invited to call the undersigned at the number provided below.

Respectfully submitted,

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